terol fed cockerels. Cir. Res. 13: 246–252.

Pihl, A., 1955. On the mode of action of fatty acids in cholesterol absorption. Acta. Physiol. Scand. 34: 197–205.

Siperstein, M. D., I. L. Chaikoff and W. O. Reinhardt, 1952. C^{14} cholesterol. 5. Obligatory function of bile in intestinal absorption of cholesterol. J. Biol. Chem. 198: 111–114.

Swell, L., D. F. Flick, H. Field, Jr. and C. R. Treadwell, 1955. Role of fat and fatty acid in absorption of dietary cholesterol. Am. J. Physiol. 180: 124–128.

Wood, J. D., J. Biely and J. E. Topliff, 1961. The effect of diet, age and sex on cholesterol metabolism in White Leghorn chickens. Canadian J. Biochem. Physiol. 39: 1705–1715.

Altered Feathering of Chicks Caused by T-2 Toxin[1]

R. D. WYATT, P. B. HAMILTON AND H. R. BURMEISTER

Department of Poultry Science and Department of Microbiology, North Carolina State University, Raleigh, N.C. 27607 and Northern Regional Research Laboratory, Peoria, Illinois 61604

(Received for publication October 1, 1974)

ABSTRACT Dietary T-2 toxin (0, 1, 2, 4, 8, and 16 μg./g.) was fed to 4 groups of 10 chickens at each treatment level from hatching until 3 weeks of age. Growth inhibitory levels (4, 8, and 16 μg./g.) caused abnormal feathering which appeared dose related. The chickens were sparsely covered with short feathers protruding at odd angles in comparison to controls. There were few feathers on the base of the neck, on the anterior dorsal surface of the wing, and on the side and back adjacent to the tail. Feather tips frequently were constricted and bent downward while the quill could have a reverse curve. A literature survey suggests that T-2 toxin may cause this effect through a nutritional imbalance.

POULTRY SCIENCE 54: 1042–1045, 1975

INTRODUCTION

T-2 TOXIN is a mycotoxin produced by several species of *Fusarium* (Burmeister *et al.*, 1972). It has been identified as 4, 15-diacetoxy-8-(3-methylbutyryloxy)-12, 13-epoxy-Δ^9-tricothecen-3-ol (Bamburg *et al.*, 1968) and like most 12,13-epoxy-Δ^9-trichothecen compounds it is a potent irritant and inflammatory agent (Marasas *et al.*, 1969). In chickens it causes severe oral lesions at dietary concentrations as low as 1 μg./g. of feed (Wyatt *et al.*, 1972b), and it has been implicated in a field outbreak of apparent fusariotoxicosis which was characterized by lesions on the feet, shanks, and heads as well as the mouth (Wyatt *et al.*, 1972a). It also causes unique disturbances of the nervous system (Wyatt *et al.*, 1973a) and other systemic effects which may not be directly related to its inflammatory properties (Wyatt *et al.*, 1973b). During these studies we have consistently observed abnormal feathering in young chickens given dietary T-2 toxin. The purpose of the present communication is to provide a brief description of the abnormal feathering and to comment on some of its implications.

MATERIALS AND METHODS

T-2 toxin was produced by growing *F. tricinctum* NRRL 3299 on white corn grits. It was extracted and purified by the method of Burmeister (1971) to give a crystalline product melting at 150 to 152° C. One-day old male chickens of a commercial broiler strain were housed in electrically heated batteries where feed and water were available

1. Published with the approval of the Director of the North Carolina State University Agricultural Experiment Station.

TABLE 6.—*Source of cholesterol in blood, liver and aortic tissues*

	Blood[1] % rec.[2]		Liver % rec.		Aorta % rec.	
	100 ml.	%	100 gm.	%	100 gm.	%
Endogenous (C^{14})	1.94 [3]	58	12.13	64	1.52	75
Exogenous (H^3)	1.40	42	7.18	36	0.52	25

[1] Blood plasma.
[2] % of initial dose recovered per 100 mls.
[3] Any two means not connected by the same line are significantly different ($P < .05$).

TABLE 7.—*Source of origin of cholesterol and cholesterol esters*

	Cholesterol % rec.[1]		Cholesterol esters % rec.	
	100 gm.	%	100 gm.	%
Exogenous (H^3)	3.43 [2]	56	7.62	73
Endogenous (C^{14})	2.64	44	2.78	27

[1] % of initial dose recovered per 100 grams of tissue.
[2] Any means not connected by the same line are significantly different ($P < .05$).

in the various tissues shows that in each case there was more endogenous than exogenous cholesterol (Table 6); however, these differences were not of significant magnitude to be statistically different in plasma. In view of the very rudimentary lymphatic system in the chicken, large quantities of dietary cholesterol are transported in both the free and esterified form. Since three-fourths of the cholesterol found in the aortic tissue was of endogenous origin, it is suggested that dietary cholesterol, in all probability, plays a rather small role in atherosclerosis in the chicken and that perhaps acetate or energy metabolism should be investigated as a major factor in this disease.

The portion of cholesterol and cholesterol esters in all tissues collectively, according to their source of origin is given in Table 7. It should be noted that approximately one-half of the free cholesterol in the tissues is of endogenous origin while almost three-fourths of the cholesterol esters are obtained from dietary sources. One-half of the plasma cholesterol, 64 percent esterified, is of endogenous origin. The liver data reveal 72 percent cholesterol occurs in the free form and 64 percent is of endogenous origin. Aortic tissue follows a similar pattern with 72 percent free cholesterol and three-fourths of this being endogenous.

It is possible that the results observed in Table 2 (free cholesterol) and that observed in Table 4 (total cholesterol) were markedly influenced by the fact that more exogenous cholesterol appears in the esterified form than in the free form. This might explain the observed deviations between the data presented in Tables 2 and 7.

REFERENCES

Bollman, J. L., and E. V. Flock, 1951. Cholesterol in intestinal and hepatic lymph in the rat. Am. J. Physiol. 164: 480–485.

Borgstrom, B., B-A. Lindhe and P. Wlodawer, 1958. Absorption and distribution of cholesterol-4-C^{14} in the rat. Proc. Soc. Exp. Biol. Med. 99: 365–368.

Cook, R. P., 1958. Cholesterol. Academic Press, Inc. New York.

Folch, J., M. Lees and G. H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497–509.

Pick, R., L. N. Katz, D. Century and P. J. Johnson, 1963. Group isolation and atherosclerosis in choles-

ad libitum under constant lighting. The feed was a commercial broiler-starter diet free of all medications. T-2 toxicosis was induced by incorporating into small portions of the diet known amounts of crystalline T-2 toxin dissolved in 50% (v./v.) aqueous ethanol. This treated feed was dried at 100° C before being mixed thoroughly into the remainder of the feed. Four replicates of 10 birds at each dose level were fed the broiler-starter diet containing 0, 1, 2, 4, 8, and 16 μg. of T-2 toxin per g. for a period of 3 weeks after hatching.

RESULTS

The feathering abnormalities occurred only at 4, 8 and 16 μg./g. which were the growth inhibitory levels, and their severity appeared to be dose related. However, not every bird was affected to the same extent and there was no readily apparent way of quantitating the feather abnormalities. Nevertheless, T-2 toxin consistently caused feather abnormalities in 3 different commercial broiler strains. Fig. 1 shows a top view of a control bird and a bird fed T-2 toxin (8 μg./g.). The bird fed T-2 toxin is sparsely covered and short

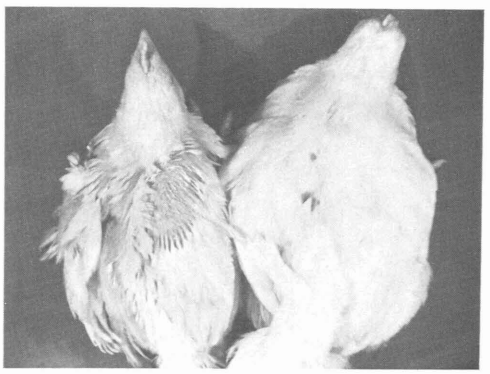

FIG. 1. Top view of a bird fed T-2 toxin (left) and a control bird (right).

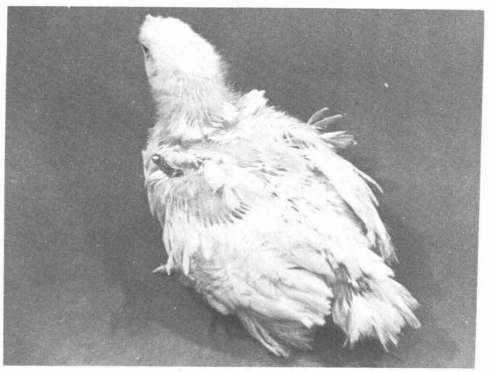

FIG. 2. View of a bird fed T-2 toxin exhibiting typical shortened tail feathers.

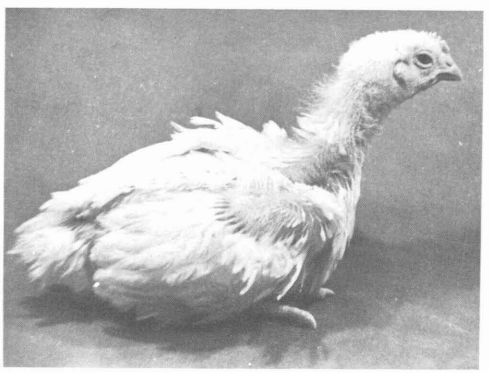

FIG. 3. Lateral view of common feathering patterns in a bird fed T-2 toxin.

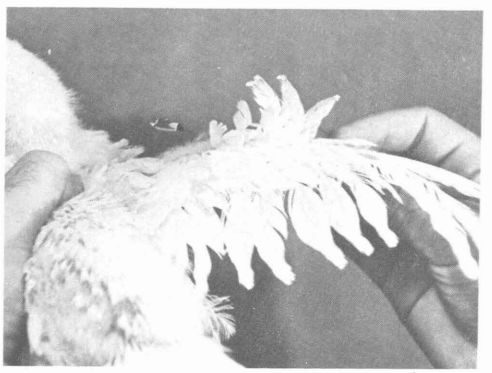

FIG. 4. Posterior view of wing feathers of a bird fed T-2 toxin.

feathers protrude from the bird at odd angles in comparison to the control bird which is fully and smoothly feathered. Fig. 2 shows another top view of a bird fed 8 μg./g. In addition to the features seen in Fig. 1, the short tail feathers are readily apparent. Fig. 3 indicates a common feather pattern. There are few feathers on the base of the neck or on the anterior dorsal surface of the wing. Less apparent is a comparative paucity of feathers on the side and back adjacent to the tail. More detail of some of the feather abnormalities are illustrated in Fig. 4. Tips of feathers frequently are constricted and bent downward. In addition, the quill may have a curve that is the reverse of a normal feather. This is particularly apparent in feathers on the anterior surface of the wing.

DISCUSSION

Abnormal feathering can be caused by genetic factors or nutritional imbalances. The extent and rapidity of feathering in chickens is controlled by specific genes (Somes, 1970). Nutrition appears to be the most important factor affecting phenotypic expression in chickens. Donaldson *et al.* (1955) reported poor feather condition at market age in broilers on a diet with a high energy to protein ratio. Leong *et al.* (1955) also reported poor feathering in New Hampshire-White Leghorn crossbred chickens on a high energy diet that was corrected by increasing the protein level of the diet. Specific amino acid deficiencies cause abnormal feathering. Arginine deficiency caused a feathering syndrome (Sanders *et al.*, 1950) similar to that described here. Leucine deficiency (Klain *et al.*, 1960b) and glycine deficiency (Hegsted *et al.*, 1941) caused twisted and ruffled feathers. A lysine deficiency coupled with excess intake of other amino acids caused feather achromatosis in Barred Plymouth Rocks (Klain *et al.*, 1960a). Magruder *et al.* (1952) found that the faulty feather structure caused by a "stress" ration containing 70% soybean oil meal was not corrected by vitamin therapy. Supplee (1966) found that a diet deficient in vitamin E and selenium caused shrunken and discolored quills of the flight feather of poults and in a few instances feathers from the tail and breast were similarly affected. A similar condition has been observed in the wing feathers of chicks deficient in pyridoxine (Daghir and Balloun, 1963; Gehle and Balloun, 1965). Waibel *et al.* (1969) observed that biotin supplementation of a deficient diet decreased the incidence of broken feathers. Zinc deficiency causes an altered wing feathering (Supplee *et al.*, 1961) similar to that caused by T-2 toxin but with zinc deficiency the body feathers appear normal.

The abnormal feathering caused by T-2 toxin appears unique in the type of abnormality and in the involvement of feathers over the entire body. These characteristics should be of diagnostic value in T-2 toxicosis which like other mycotoxicoses is characterized by reduced growth rate. In addition, it can add to the extensive list of abnormalities caused by mycotoxins in chickens. Presently, one may only speculate on the mechanism by which T-2 toxin causes altered feathering, but the literature strongly suggests that the metabolism of essential nutrients is impaired. A more direct effect on the follicular tissue where the feathers are formed appears also to be an attractive hypothesis. At any rate, this feather abnormality offers a new point of attack in gaining an understanding of the complex syndrome of T-2 toxicosis.

REFERENCES

Bamburg, J. R., N. R. Riggs and F. M. Strong, 1968. The structures of toxins from two strains of *Fusarium tricinctum*. Tetrahedron, 24: 3329–3336.

Burmeister, H. R., 1971. T-2 toxin production by *Fusarium tricinctum* on solid substrate. Appl. Microbiol. 21: 739–742.

Burmeister, H. R., J. J. Ellis and C. W. Hesseltine, 1972. Survey for fusaria that elaborate T-2 toxin.

Appl. Microbiol. 23: 1165–1166.

Daghir, N. J., and S. L. Balloun, 1963. Evaluation of the effect of breed on vitamin B_6 requirements of chicks. J. Nutr. 79: 279–288.

Donaldson, W. E., G. F. Combs, G. L. Romoser and W. C. Supplee, 1955. Body composition, energy intake, feed efficiency, growth rate, and feather condition of growing chickens as influenced by calorie-protein ratio of the ration. Poultry Sci. 34: 1190.

Gehle, M. H., and S. L. Balloun, 1965. Selected hemocytological effects of vitamin B_6 deficiency in chicks. J. Nutr. 87: 197–201.

Hegsted, D. M., G. M. Briggs, C. A. Elvejhem and E. B. Hart, 1941. The role of arginine and glycine in chick nutrition. J. Biol. Chem. 140: 191–200.

Klain, G. J., D. C. Hill, J. A. Gray and E. M. Olson, 1960a. Observations on the influence of dietary protein level and amino acid balance on pigmentation in the feathers of chicks. Poultry Sci. 39: 25–29.

Klain, G. J., H. M. Scott and B. C. Johnson, 1960b. The amino acid requirement of the growing chick fed a crystalline amino acid diet. Poultry Sci. 39: 39–44.

Leong, K. C., M. L. Sunde, H. R. Bird and C. A. Elvehjem, 1955. Effect of energy: protein ratio on growth rate, efficiency, feathering, and fat deposition in chickens. Poultry Sci. 34: 1206–1207.

Magruder, N. D., J. K. Bletner, T. B. Clark, C. E. Weakley and A. H. Van Landingham. 1952. The variable response of normal chicks to "stress" rations and a vitamin B_{12} supplement. Poultry Sci. 31: 925.

Marasas, W. F. O., J. R. Bamburg, E. B. Smalley, F. M. Strong, W. L. Ragland and P. E. Degurse, 1969. Toxic effects on trout, rats and mice of T-2 toxin produced by the fungus *Fusarium tricinctum* (Cd.) Synd. Hans. Toxicol. Appl. Pharmacol. 15: 471–482.

Sanders, B. G., S. O. Brown and J. R. Couch, 1950. A feathering syndrome in chicks after feeding optimal levels of lysine in absence of arginine. Proc. Soc. Expt. Biol. Med. 74: 114–117.

Somes, R. G., 1970. The influence of the rate of feathering allele K^n on various quantitative traits in chickens. Poultry Sci. 49: 1251–1256.

Supplee, W. C., 1966. Feather abnormality in poults fed a diet deficient in vitamin E and selenium. Poultry Sci. 45: 852–854.

Supplee, W. C., R. D. Creek, G. F. Combs and D. L. Blamberg, 1961. The zinc requirements of poults receiving practical diets. Poultry Sci. 40: 171–176.

Waibel, P. E., L. M. Krista, R. L. Arnold, L. G. Blaylock and L. H. Neagle, 1969. Effect of supplementary biotin on performance of turkeys fed corn-soybean meal type diets. Poultry Sci. 48: 1979–1985.

Wyatt, R. D., W. M. Colwell, P. B. Hamilton and H. R. Burmeister, 1973a. Neural disturbances in chickens caused by dietary T-2 toxin. Appl. Microbiol. 26: 757–761.

Wyatt, R. D., P. B. Hamilton and H. R. Burmeister, 1973b. The effects of T-2 toxin in broiler chickens. Poultry Sci. 52: 1853–1859.

Wyatt, R. D., J. R. Harris, P. B. Hamilton and H. R. Burmeister, 1972a. Possible outbreaks of fusariotoxicosis in avians. Avian Dis. 16: 1123–1130.

Wyatt, R. D., B. A. Weeks, P. B. Hamilton and H. R. Burmeister, 1972b. Severe oral lesions in chickens caused by ingestion of dietary fusariotoxin T-2. Appl. Microbiol. 24: 251–257.

NEWS AND NOTES

(Continued from page 1030)

HUBBARD MILLING NOTES

Dr. Richard C. Eaton has been appointed Vice President and Manager of the Feed Division of the Hubbard Milling Company, Mankato, Minnesota. He replaces Paul J. Frederick who will assume the position of President and Chief Executive Officer of Springfield Milling Corporation, a subsidiary of Hubbard Milling. For the past 2-1/2 years, Eaton has been President of a Monsanto subsidiary, Farmers Hybrid Companies, Inc., Hampton, Iowa. Prior to 1972, Eaton was associated with Ralston Purina Company.

CONRAD NOTES

Dr. Robert J. Mackin has joined Conrad's Poultry Farm and Hatchery, Inc., Goodells, Michigan, as Director of Technical Service and Marketing. Prior to joining Conrad's, a franchise distributor of Babcock B-300, Mackin was with Babcock Poultry Farm in

(Continued on page 1053)

Water Balance of the Hen During Egg Formation

B. R. HOWARD[1]
Agricultural Research Council's Poultry Research Centre, West Mains Road, Edinburgh, EH9 3JS

(Received for publication October 1, 1974)

ABSTRACT Laying hens consume more water on days when an egg is laid, than on non-laying days; the daily water intake is more than double—from 115 g. to 255 g. and the number of drinks taken increases proportionately. The weight of water in the egg produced is only 32 g. The requirement for this excess of water implies a considerable metabolic stress associated with egg formation. Studies have been carried out to determine why this water is consumed, and its metabolic fate.

Water ingestion started to increase about 12 h. before oviposition; it rose steadily until about 2 h. before lay, then fell sharply. Urine minute volume showed similar fluctuations. At the time when the urine output fell just before lay, plasma osmolarity was higher than usual; this might be predicted from the drop in water consumption. Alterations in the water content of the oviduct were not sufficient to explain the increased fluid intake, and total body weight remained constant in spite of the consumption of the additional 140 g. water. These findings suggest that the water has a metabolic role, as it is not retained as a net fluid surplus.

POULTRY SCIENCE 54: 1046–1053, 1975

INTRODUCTION

THE formation of an egg involves the formation and transportation of considerable quantities of protein and minerals across the wall of the oviduct. In part, at least, these materials are derived from the increase in food consumption during this period (Morris and Taylor, 1967). The increased demands for raw materials require additional measures for transportation and dictate that fluid ingestion should also be increased.

Jull (1949) commented on the fact that during the first year of production a laying flock of hens consumed over 4 kg. additional water for each dozen eggs produced. Later, Lifschitz *et al.* (1967) reported that White Leghorn hens ingested almost twice as much water per day as cockerels. They also reported that there was a sharp reduction in fluid intake immediately before oviposition; drinking activity rose again subsequently.

These findings were confirmed by Anderson and Hill (1967), who also pointed out that in pre-lay and laying hens food and water intake were linearly related. When the supply of food was restricted, the pattern of water intake was not correspondingly altered (the converse situation was also true); it is clear that under these conditions, food and water are independent of one another.

Chapman and Black (1967), using radioisotope techniques, demonstrated that laying hens had a higher water turnover than cockerels, although this did not appear to be related to oviposition. This observation was extended by Chapman and Mihai (1972), who reported that in non-laying hens the rate of water turnover was higher than in cockerels, but that during lay the rate of turnover increased further and was associated with a rise in the size of the total body water pool from 54 to 62% of body weight. Additional evidence for the importance of water during development of the egg was presented by Ballard and Bieller (1969) and Wood-Gush and Horne (1970) who showed that in Leghorn hens both feed and water intake rose before oviposition, but fell immediately afterward.

It seems probable that important changes in water balance occur in the laying hen, and that some of these at least can be related to oviposition. The experiments presented

1. Present Address: Department of Animal Biology, School of Veterinary Medicine, Pahlavi University, Shiraz, Iran.